



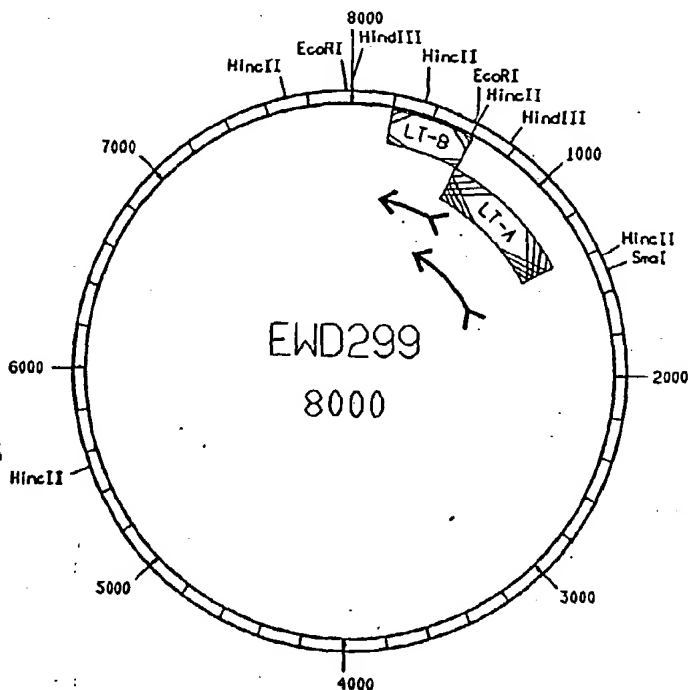
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<p>(21) International Application Number: PCT/EP92/03016</p> <p>(22) International Filing Date: 30 December 1992 (30.12.92)</p> <p>(30) Priority data: MI91A03513 31 December 1991 (31.12.91) IT</p> <p>(71) Applicant (for all designated States except US): BIOCINE SCLAVO SPA [IT/IT]; Via Fiorentina, 1, I-53100 Siena (IT).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): DOMENIGHINI, Mario [IT/IT]; Via Ungaretti, 17, I-53010 Quercegrossa (IT). RAPPUOLI, Rino [IT/IT]; Via Calamandrei, 39, I-53010 Quercegrossa (IT). PIZZA, Mariagrazia [IT/IT]; Via Colombini, 30, I-43100 Siena (IT). HOL, Wim [NL/US]; 18332 57th Avenue, N.E., Seattle, WA 98155 (US).</p>		<p>(74) Agent: HALLYBONE, Huw, George; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).</p> <p>(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: **IMMUNOGENIC DETOXIFIED MUTANTS OF CHOLERA TOXIN AND OF THE TOXIN LT, THEIR PREPARATION AND THEIR USE FOR THE PREPARATION OF VACCINES**

(57) Abstract

An immunogenic detoxified protein comprising the amino acid sequence of subunit A of cholera toxin (CT-A) or subunit A of an *Escherichia coli* heat labile toxin (LT-A) or a fragment thereof wherein one or more amino acids at, or in positions corresponding to Val-53, Ser-63, Val-97, Tyr-104 or Pro-106 are replaced with another amino acid or deleted. Examples of specific replacements include Val-53-Asp, Val-53-Glu, Val-53-Tyr, Ser-63-Lys, Val-97-Lys, Val-97-Tyr, Tyr-104-Lys, Tyr-104-Asp, Tyr-104-Ser, Pro-106-Ser. The immunogenic detoxified protein is useful as vaccine for *Vibrio cholerae* or an enterotoxigenic strain of *Escherichia coli* and is produced by recombinant DNA means by site-directed mutagenesis.



LT2	1	-.FF---T---R-A---L---QQ-AYE---PI---	38
LT1	1	-----FRS-----	39
LT1_1A	1	-G-R-----R-----HN-----	40
CT	1	NDDKLYRADSRPPDEIKQSGGLMPRGQSEYFDRGTQMNIN	40
		-----E-----V--NT--N-----TVT--Q---I--N--GS-	78
		-----Y-----	79
		-----Y-----L-----A--S---Y	80
		LYDHARGTQTGFVRHDDGYVST ¹ ISLRS ² AHLVGQTILSGH	80
		NE-----V-P---L-D--G---R---Y-S-N-FA-----	118
		-LTIYI-----IS-----	116
		-----V---Y-----	120
		STYYIYVIATAPNMFN ³ VNDVLGAYSPHPDEQEVSALGGIP	120
		L---I-----SF-A-EGGMQ---D--GDLF-G-TV--N--	158
		-----	156
		-----N---I--R-----E-----R--N---E-	160
		YSQIYGWYRVHFGVLDEQLHRNRYRDRYYSNLDIAPAD	160
		--Q-----SNFP---M--STF--EQ-VPNNKEFK-GV-I	198
		-----	196
		--R-----D-Q-----Q---DSS-TITGD--N	200
		GYGLAGFPPEHRAWREEPWIHHAPPGCGNAPRSSMSNTCD	200
		SA-NV--KYD-MNFKKLL--RLALTFFM--D-F-GVHGE----	241
		-----	236
		-E--N-STIY-R-----D---EV-.IY---.R---	240
		EKTQSLGVKFLDEYQSKVKRQIFSGY.QSDID.THNRI.KDEL	240

Figure 1